

# Serotonin<sub>1B</sub> heteroreceptor activation induces an antidepressant-like effect in mice with an alteration of the serotonergic system

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**Objective:** We sought to demonstrate whether the specific activation of serotonin<sub>1B</sub> (5-HT<sub>1B</sub>) heteroreceptors by systemic or local administration of the selective 5-HT<sub>1B</sub> receptor agonist anpirtoline could mediate antidepressant-like effects in mice. **Methods:** We confirmed the selectivity of action of anpirtoline in the forced swim test (FST) in 5-HT<sub>1B</sub> knockout mice. We then evaluated the behavioural effects of anpirtoline on 5-HT-lesioned (5,7-dihydroxytryptamine creatinine [5,7-DHT]) and 5-HT-depleted (*p*-CPA) mice. We estimated the depletion level and selectivity of action of 5,7-DHT and *p*-CPA by measuring the neurotransmitter levels and [<sup>3</sup>H]-citalopram binding. We investigated the antidepressant-like effect of anpirtoline when locally perfused in an area of the brain where the response is mainly attributable to presynaptic (cortex and hippocampus) or postsynaptic receptors (substantia nigra and caudate putamen). Furthermore, we evaluated the effect of the 5-HT<sub>1B</sub> receptor antagonist GR127935 on the activity of various antidepressants in the FST. **Results:** Anpirtoline was devoid of effects in 5-HT<sub>1B</sub> receptor knockout mice. It induced a greater effect in *p*-CPA and 5,7-DHT pretreated mice compared with control subjects, suggesting that the antidepressant-like activity of anpirtoline mainly depends on 5-HT<sub>1B</sub> heteroreceptor stimulation (autoreceptors being destroyed by 5,7-DHT). This observation was confirmed by the results showing the antidepressant-like effect of anpirtoline when locally perfused in areas of the brain that contain postsynaptic receptors. The blockade of 5-HT<sub>1B</sub> receptors antagonizes the effect of selective serotonin reuptake inhibitors (SSRIs). **Conclusion:** Our results demonstrate that the antidepressant-like effect of SSRIs in the FST requires the activation of 5-HT<sub>1B</sub> heteroreceptors.

**Objectif :** Nous avons cherché à démontrer si l'activation spécifique des hétérorécepteurs sérotoninergiques de type IB (5-HT<sub>1B</sub>) par l'administration systémique ou locale d'un agoniste sélectif des récepteurs 5-HT<sub>1B</sub>, l'anpirtoline, pouvait induire un effet de type antidépresseur chez les souris. **Méthodes :** Nous avons confirmé la sélectivité d'action de l'anpirtoline au cours du test de la nage forcée (FST) chez des souris invalidées pour le gène codant pour le récepteur 5-HT<sub>1B</sub>. Nous avons ensuite évalué les effets comportementaux de l'anpirtoline chez des souris avec une lésion 5-HT (5,7-dihydroxytryptamine créatinine [5,7 DHT]) ou une déplétion (*p*-CPA) du système 5-HT. Nous avons évalué le taux de déplétion et la sélectivité d'action de la 5,7-DHT et du *p*-CPA en mesurant les concentrations de neurotransmetteurs et la liaison [<sup>3</sup>H]-citalopram au transporteur 5-HT. Nous avons étudié l'effet de type antidépresseur d'une perfusion locale d'anpirtoline dans des régions du cerveau où la réponse est attribuable principalement aux récepteurs présynaptiques (cortex et hippocampe) ou postsynaptiques (substance noire et putamen caudé). De plus, nous avons évalué l'effet d'un antagoniste des récepteurs 5-HT<sub>1B</sub>, le GR127935, sur l'activité de divers antidépresseurs dans le FST. **Résultats :** L'anpirtoline n'avait pas d'effet chez les souris. L'anpirtoline a produit plus d'effet chez les souris préalablement traitées avec le *p*-CPA et la 5,7-DHT comparativement aux sujets témoins. Ce résultat

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montre que l'activité de type antidépresseur de l'anpirtoline dépend principalement de la stimulation des hétérorécepteurs 5-HT<sub>1B</sub> (les autorécepteurs étant détruits par la 5,7-DHT). Cette observation a été confirmée par les résultats montrant que l'anpirtoline a un effet de type antidépresseur lors d'une perfusion locale dans des régions du cerveau contenant des récepteurs postsynaptiques. Le blocage des récepteurs 5-HT<sub>1B</sub> antagonise l'effet des inhibiteurs sélectifs du recaptage de la sérotonine (ISRS). **Conclusion** : Nos résultats démontrent que l'activation des hétérorécepteurs 5-HT<sub>1B</sub> est nécessaire pour observer un effet de type antidépresseur des ISRS dans le FST.

## Introduction

It has been repeatedly demonstrated through the use of intracerebral *in vivo* microdialysis that the absence of serotonin<sub>1B</sub> (5-HT<sub>1B</sub>) receptors in knockout mice, or its blockade by a selective 5-HT<sub>1B</sub> receptor antagonist GR127935, potentiates the increase of the extracellular serotonin levels ([5-HT]<sub>EC</sub>) induced by a single intraperitoneal administration of selective serotonin reuptake inhibitors (SSRIs).<sup>1-3</sup> These data suggest that the activation of 5-HT<sub>1B</sub> autoreceptors limits the effects of SSRIs on dialysate 5-HT levels at serotonergic nerve terminals, particularly in the hippocampus and the frontal cortex. Surprisingly, this increase in [5-HT]<sub>EC</sub> did not correlate with an increase of the antidepressant-like activity evaluated in the forced swim test (FST) in mice.<sup>4</sup> In contrast, it has been reported that a decrease in immobility time induced by SSRIs in the FST was absent in 5-HT<sub>1B</sub> knockout mice and blocked by GR127935 in wild-type mice, which suggests that the activation of 5-HT<sub>1B</sub> receptors may mediate the antidepressant-like effects of SSRIs. We hypothesized that the antidepressant-like effects of 5-HT<sub>1B</sub> receptor agonists,<sup>5,6</sup> and probably those of SSRIs, might be mediated by the activation of 5-HT<sub>1B</sub> heteroreceptors. These heteroreceptors are located on nonserotonergic neurons, particularly on dopaminergic, glutamatergic, cholinergic and GABAergic neurons.<sup>7</sup>

A FST in mice demonstrated recently that dopamine (DA) depletion was associated with a loss of antidepressant-like activity of the SSRIs citalopram and paroxetine, suggesting that the antidepressant-like effect of SSRIs observed in the FST in mice requires an activation of DA pathways.<sup>8</sup> Interestingly, numerous microdialysis studies also reported an increase in DA release following systemic or local administration of SSRIs or 5-HT<sub>1B</sub> receptor agonists in the substantia nigra,<sup>9</sup> striatum,<sup>10,11</sup> nucleus accumbens,<sup>12</sup> frontal cortex<sup>11</sup> and the ventral tegmental area (VTA).<sup>13</sup> Furthermore, many studies have shown that the systemic administration of subactive doses of 5-HT<sub>1B</sub> receptor agonists such as anpirtoline and RU 24969 can potentiate the effect of antidepressants in the FST in mice.<sup>14,15</sup>

Taken together, these findings suggest that 5-HT and DA systems interact closely and influence the antidepressant-like effect of SSRIs in the FST in mice. However, the details of such a connection are not yet known.

We sought to determine whether the specific activation of 5-HT<sub>1B</sub> heteroreceptors by the systemic or local administration of the 5-HT<sub>1B</sub> receptor agonist anpirtoline could mediate antidepressant-like effects in mice, similar to the results obtained with SSRIs.<sup>5,6</sup>

## Methods

### Animals

The wild-type (129/Sv) and mutant colonies that we used were the product of heterozygous matings at the animal facility of Columbia University. These mice were then shipped to France, and their offspring were bred and reared in independent colonies as described previously.<sup>3</sup> We obtained wild-type and 5-HT<sub>1B</sub> knockout mice from a pure 129/Sv genetic background. We also used male Swiss mice (Centre d'élevage Janvier, Le Genest, France) aged 4 weeks and weighing 18–20 g at the time of treatment.

We housed the animals in groups of 18 per cage (40 × 28 × 17 cm) on a 12-hour light/dark cycle (lights on between 7 am and 7 pm), and the mice had free access to food and water. We maintained the ambient temperature of the room at 21°C (standard deviation [SD] 1°C), and the humidity was 50%. Each experimental group consisted of naive, randomly grouped mice of the same weight, which we used only once. We performed all experiments between 7 am and 12 pm within the guidelines of the French Ministry of Agriculture for experiments with laboratory animals (law 87 848, permission no. 44-012B to M.B.).

### Drugs

We dissolved a range of doses of paroxetine (4–16 mg/kg; GlaxoSmithKline), citalopram (4–16 mg/kg; Lundbeck), desipramine (8–32 mg/kg; Sigma) and imipramine (8–32 mg/kg; Sigma-Aldrich) in distilled water and administered them intraperitoneally (25 mL/kg). We dissolved GR127935 (4 mg/kg; GlaxoSmithKline) in distilled water and injected it subcutaneously (25 mL/kg) 45 minutes before the FST. We administered the antidepressants 15 minutes after the GR127935 injection (i.e., 30 min before FST). We dissolved anpirtoline (Tocris Bioscience) in artificial cerebrospinal fluid, which we then locally perfused (flow rate of 0.2 µL/min). We performed serotonergic lesion with 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT; Sigma-Aldrich) administered intracerebroventricularly, whereas we performed serotonergic depletion with parachlorophenylalanine methyl-ester (*p*-CPA, Sigma-Aldrich) at a dose of 300 mg/kg administered intraperitoneally each day for 3 consecutive days (72, 48 and 24 h before the test).

### Surgical procedures

#### Serotonergic lesion

We pretreated all mice 30 minutes before the intracerebroventricular administration of 5,7-DHT (or saline) with intra-

peritoneal injections of 20 mg/kg of desipramine HCl (Ciba) and 25 mg/kg of nomifensine maleate (Ciba) to protect the neurons containing norepinephrine and DA, respectively.<sup>16</sup> We then administered anesthesia intraperitoneally (400 mg/kg of chloral hydrate) and placed the mice in a stereotaxic instrument (ASI) fitted with atraumatic earbars. We injected 5,7-DHT dissolved in 0.1% ascorbic acid using an infusion pump (KD Scientific) at a flow rate of 0.2  $\mu$ L/min for 7.5 minutes, and we left the needle in place for another 2 minutes to prevent efflux of the injected solution. Treated mice received a bilateral intracerebroventricular injection of 11.4  $\mu$ g of 5,7-DHT (free base) with the following coordinates from bregma (in mm): anteroposterior (AP) -0.7, lateral (L)  $\pm$  1, ventral (V) 3.5. After surgery, we allowed a post-operative period of 14 days to obtain degeneration of neurons containing 5-HT (delay of action of neurotoxin<sup>16</sup>).

After these 14 days, we sacrificed 2 groups of treated mice by cervical dislocation to determine the 5-HT depletion levels and the selectivity of action of the neurotoxin (brain tissue dosage of neurotransmitters by high-performance liquid chromatography and [<sup>3</sup>H]-citalopram binding). We used 2 other groups of mice for behavioural studies (locomotor activity and FST). We confirmed the 5-HT depletion level by high-performance liquid chromatography, which we performed the day after the test.

#### Local infusion of anpirtoline

After administering chloral hydrate, we placed the mice in an ASI stereotaxic instrument. We implanted guide cannulae (internal diameter 0.35 mm, external diameter 0.60 mm; Unimed) stereotaxically in the following brain areas: coordinates from bregma (in mm) were AP +1.0, L  $\pm$  2.0, V 1.7 for the prefrontal cortex; AP -1.7, L  $\pm$  1.0, V 1.5 for the ventral hippocampus; AP +0.0, L  $\pm$  2.0, V 3.0 for the caudate putamen and AP -3.3, L  $\pm$  1.5, V 4.5 for the substantia nigra. We secured the guide cannulae with dental cement (GC Europe). After 7 days of recovery, we lowered the injection cannulae (internal 0.15 mm, external diameter 0.30 mm; Unimed) into the guide cannulae in freely moving mice. We locally perfused anpirtoline at a flow rate of 0.2  $\mu$ L/min for 2 minutes using a PHD 2000 infuse pump (Harvard Apparatus). We then removed the cannulae and immediately began the FST in the mice.

At the end of the experiment, we killed the mice and quickly removed their brains, which we froze in isopentane at -30°C and stored at -80°C. We determined the exact location of the guide cannulae using a digital photomicrograph of coronal sections of frozen brain.<sup>17</sup> We discarded data when the guide cannulae were not located in the targeted area of the brain.

#### Determination of depletion level and selectivity of action of 5,7-DHT

To prepare samples and perform high-performance liquid chromatography analysis, we killed the mice by cervical dislocation without anesthesia. We rapidly removed the brain from the cranium and dissected it on a cooled aluminum apparatus, as described previously.<sup>8</sup> We weighted the sections

of the brain (cortex and hippocampus) into a 1.5-mL polypropylene tube. We added 600  $\mu$ L (for tubes containing the hippocampus) and 1200  $\mu$ L (for tubes containing the cortex) of an acid solution (8.8 mg of ascorbic acid and 122 mg of ethylenediaminetetraacetic acid (EDTA) in 1000 mL of perchloric acid 0.1 M) to each tube. We then disrupted the tissue by sonication, and we centrifuged the solution at 12 000 g for 10 minutes at 4°C. We stored the supernatant at -80°C. The preparation of samples and the high-performance liquid chromatography analysis have been fully described in a recent methodologic article.<sup>18</sup>

To perform autoradiographic studies, we sacrificed the mice, and we quickly removed the brains, which we froze in isopentane at -30°C and stored at -80°C. We cut coronal 20  $\mu$ m tissue sections in a cryostat at -20°C (Microm HM 560) throughout the hindbrain, and we thaw-mounted the sections onto chrome-alum slides coated with 5% gelatine. We dried the slides, which we stored dessicated at -80°C until we were ready to use them.<sup>19</sup> Then, we performed binding of [<sup>3</sup>H]-citalopram on the 5-HT neuronal transporter. Before autoradiographic experiments, we thawed sections of the brain under vacuum for 20 minutes and stored them at room temperature until incubation. We performed autoradiographic studies with ligand on adjacent slices from the same animals.

#### [<sup>3</sup>H]-citalopram binding<sup>20</sup>

We preincubated slides for 15 minutes at room temperature in Tris buffer (Tris HCl 50 mM, NaCl 120 mM, KCl 5 mM; pH 7.4). To assess total binding, we performed the incubation at room temperature for 1 hour in Coplin jars containing 30 mL for 10 slides of the same buffer with 1 nM [<sup>3</sup>H]-citalopram (specific activity 84.2 Ci/mmol; Perkin Elmer). We evaluated nonspecific binding on adjacent sections incubated in the same conditions plus 10<sup>-5</sup> M fluoxetine (Lilly). Then, we dipped the slides into buffer, rinsed them twice in the same buffer at 4°C for 10 minutes and dipped them in distilled water. We dried the slides under a cold air stream for 30 minutes and allowed them to dry overnight. We then opposed the sections on BioMax MR films (Kodak) for 4 weeks with tritiated standard strips (Amersham). We quantified the autoradiograms using a computerized image analysis system (TRIBVN).

Since 5-HT<sub>1B</sub> autoreceptors are located on presynaptic 5-HT nerve terminals, the decrease in [<sup>3</sup>H]-citalopram binding sites after 5,7-DHT injection, therefore, should be linked to a decrease in the number of 5-HT neurons and to a decrease in the density of 5-HT<sub>1B</sub> autoreceptors.<sup>21</sup> We analyzed results only for the cortex and hippocampus, areas of the brain containing high density of both 5-HT transporters and 5-HT<sub>1B</sub> receptors and where an augmentation of the neurobiochemical effects of SSRIs by 5-HT<sub>1B</sub> receptor antagonist has been reported.

#### Behavioural tests

##### Measurement of locomotor activity in mice<sup>22</sup>

We kept animals in the darkened test room for at least 1 hour before the test for habituation. After injection (saline or treat-

ment), we replaced the mice in their cages for the required injection–test interval and then individually transferred them to the actimeter for the 10-minute test. We recorded the spontaneous activity of naive animals using a photoelectric actimeter (OSYS Technology). This actimeter consists of a stainless steel apparatus containing transparent cages in which the animals' horizontal activity is measured by light beams connected to a photoelectric cell. We recorded their activity during a 10-minute period. We performed the actimeter test independently of the FST to examine the effect of the drugs on the spontaneous locomotor activity of mice.

### Measurement of immobility time in the FST

The FST was essentially similar to that described elsewhere.<sup>23</sup> We dropped mice individually into glass cylinders (height 25 cm, diameter 10 cm) containing 10 cm water, maintained at 23–25°C, and they remained there for 6 minutes. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above the water. We tested 6 mice simultaneously, and we recorded the time of immobility during the last 4 minutes of the 6-minute testing period (after 2 minutes of habituation). The same well-trained researchers, blinded to the treatments, performed the test in all mice. Results are expressed as the immobility time during the 240-second test period.

### Statistical analysis

We performed statistical analyses using the computer software Sigmasat (Systat Software). For behavioural tests, we performed a two-way analysis of variance (ANOVA) on either the immobility time (FST) or the number of beams broken (locomotor activity), using the 5-HT depletion (or pre-treatment or genetic background) and the drug treatment as the main factors, followed by a post hoc Newman–Keuls test. We analyzed the depletion of monoamine levels in the brain and [<sup>3</sup>H]-citalopram binding using 1-way ANOVA followed by a Newman–Keuls test. For the local infusion study, we performed one-way ANOVA (effect of anpirtoline) on the immobility time, which we followed with a Newman–Keuls test when appropriate. We considered results to be statistically significant at  $p < 0.05$ .

## Results

### Effects of anpirtoline treatment in 5-HT<sub>1B</sub> knockout and wild-type nonoperated mice in behavioural studies

The two-way ANOVA performed (mice genotype  $\times$  treatment) on the immobility time of mice in the FST revealed statistically significant effects of genotype (wild-type or 5-HT<sub>1B</sub> knockout;  $F_{1,36} = 29.29$ ,  $p < 0.001$ ) and treatment (saline or anpirtoline;  $F_{1,36} = 28.67$ ,  $p < 0.001$ ) as well as a significant interaction between these 2 factors ( $F_{1,36} = 25.11$ ,  $p < 0.001$ ). Anpirtoline (4 mg/kg) injected intraperitoneally in wild-type mice significantly decreased the mean immobility time in the FST ( $p < 0.001$ ) by 15% compared with the corresponding group that received saline. The mean (and standard error of

the mean [SEM]) in wild-type mice was 237 (2) s for those that received saline and 200 (6) s for those that received anpirtoline. A 4-mg/kg dose of anpirtoline did not alter the mean immobility time of the 5-HT<sub>1B</sub> knockout mice in the FST compared with the corresponding group that received saline (238 [SEM 1] s v. 237 [SEM 2] s,  $p = 0.810$ ). Immobility time was significantly greater in 5-HT<sub>1B</sub> knockout mice treated with anpirtoline compared with their wild-type littermates ( $p < 0.001$ ), whereas we observed no difference between knockout and wild-type mice treated with saline ( $p = 0.779$ ) (Fig. 1).

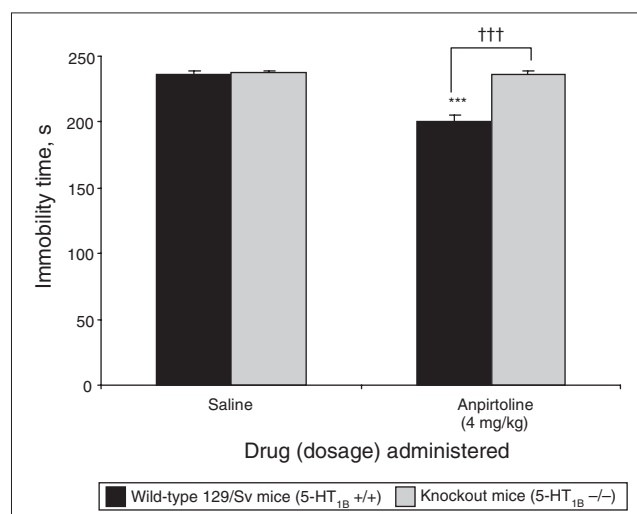
The two-way ANOVA (mice genotype  $\times$  treatment) on the locomotor activity of mice did not reveal statistically significant effects based on genotype (wild-type or 5-HT<sub>1B</sub> knockout;  $F_{1,36} = 0.37$ ,  $p = 0.545$ ) and treatment (saline or anpirtoline;  $F_{1,36} = 0.217$ ,  $p = 0.644$ ), nor did it reveal a significant interaction between these 2 factors ( $F_{1,36} = 1.141$ ,  $p = 0.293$ ) (data not shown).

These results suggest that the decrease in immobility time in the FST that we observed in wild-type mice is not linked to a psychostimulant effect of anpirtoline and that the absence of effect in knockout mice is not linked to a sedative effect of anpirtoline. Thus, the antidepressant-like effect of anpirtoline appears to be mediated by the activation of 5-HT<sub>1B</sub> receptors.

On the basis of these results, we chose doses of 4 and 8 mg/kg for the studies in sham-operated mice and those treated with neurotoxin.

### Effects of 5,7-DHT treatment in wild-type mice

The intracerebroventricular perfusion of 22.8  $\mu$ g of 5,7-DHT (freebase) per mouse induced a significant decrease in 5-HT and 5-HIAA levels in both the cortex and hippocampus (Fig. 2A). In the cortex, 5-HT levels (one-way ANOVA: lesion,  $F_{1,16} = 33.6$ ,  $p < 0.001$ ) were significantly decreased by 42.2%



**Fig. 1:** Effects of anpirtoline on immobility time in the forced swim test (FST) in wild-type 129/Sv and serotonin<sub>1B</sub> (5-HT<sub>1B</sub>) knockout mice ( $n = 10$  per group). We performed two-way analysis of variance (ANOVA) followed by a Newman–Keuls test. \*\*\*Significant difference between treatment and control groups ( $p < 0.001$ ). †††Significant effect of genotype between groups receiving the same treatment ( $p < 0.001$ ).



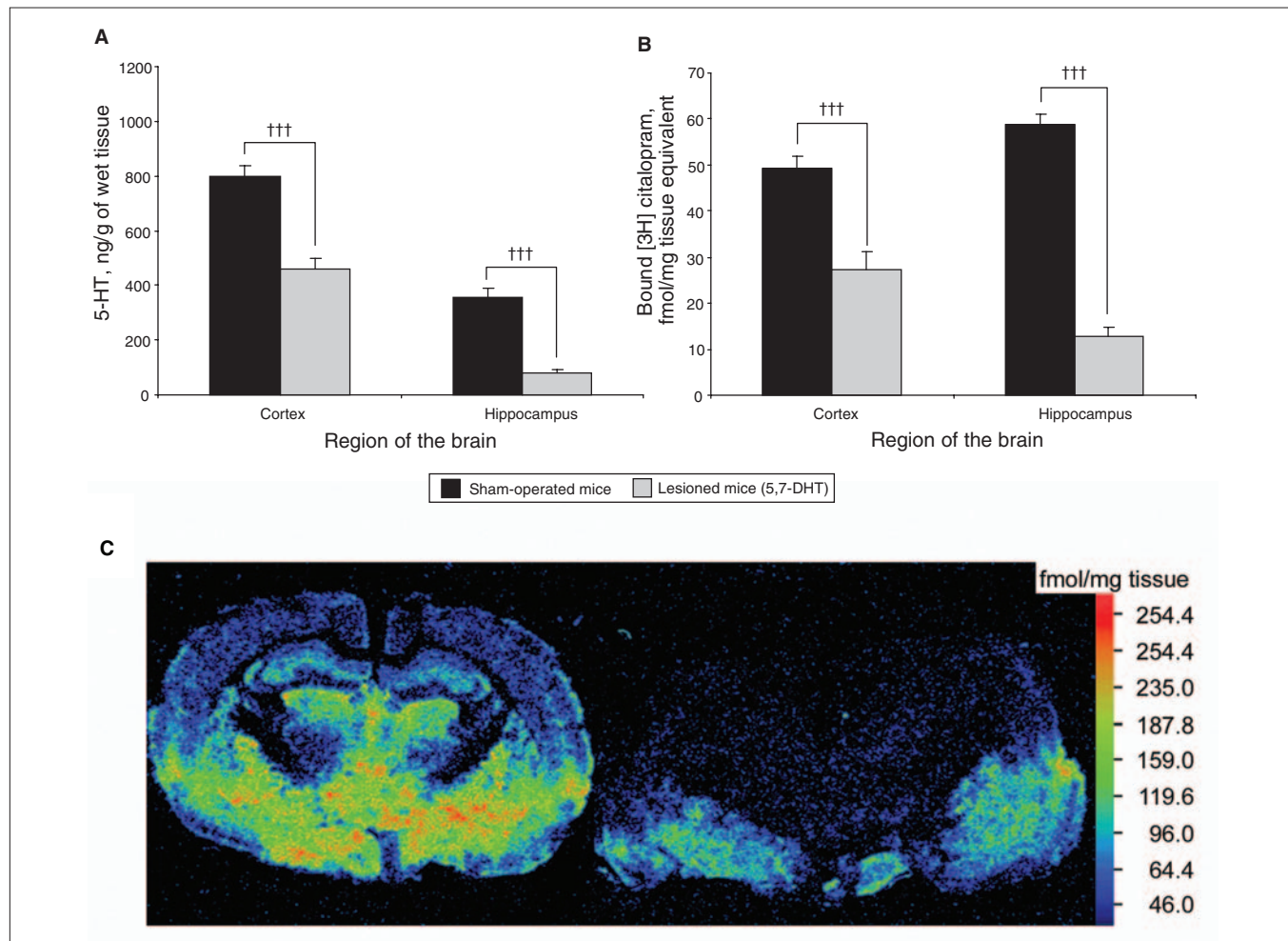
(nonlesioned 798 ng/g v. lesioned 461 ng/g of wet weight of tissue), and 5-HIAA levels were markedly reduced (one-way ANOVA: lesion,  $F_{1,16} = 33.58$ ,  $p < 0.001$ ) by 45.3% (nonlesioned 550 ng/g v. lesioned 300 ng/g of wet weight of tissue). In the hippocampus, 5-HT levels (one-way ANOVA: lesion,  $F_{1,17} = 52.51$ ,  $p < 0.001$ ) were significantly decreased by 76.5% (nonlesioned 354 ng/g v. lesioned 83 ng/g of wet weight of tissue), and 5-HIAA levels were decreased (one-way ANOVA: lesion,  $F_{1,18} = 19.96$ ,  $p < 0.001$ ) by 75.4% (nonlesioned 460 ng/g v. lesioned 113 ng/g of wet weight of tissue).

Neither the 5,7-DHT lesion in the cortex nor that in the hippocampus affected levels of other neurotransmitters ( $p > 0.05$ ; data not shown). These results indicate that the lesion was selective and restricted to the brain 5-HT systems. The brain turnover of 5-HT was not significantly different among sham-operated and 5,7-DHT-lesioned mice. The mean 5-HIAA:5-HT ratio (and SEM) in the cortex was 0.72 (0.05) for sham-operated mice compared with 0.74 (0.08) for lesioned mice; the mean ratio (and SEM) in the hippocampus was 1.43 (0.16) for sham-operated mice compared with 1.44 (0.12) for lesioned mice.

We also used an autoradiographic method with [ $^3$ H]-citalopram to determine whether 5-HT lesion was linked to nerve terminal destruction. The autoradiography data showed a 45% decrease in the number of ligand binding sites in the cortex ( $F_{1,14} = 22.53$ ,  $p < 0.001$ ; nonlesioned 49.3 fmol/mg v. lesioned 27.2 fmol/mg) and a 78% decrease in the hippocampus ( $F_{1,14} = 210.19$ ,  $p < 0.001$ ; nonlesioned 58.7 fmol/mg v. lesioned 12.9 fmol/mg) (Fig. 2b). These results indicate that the destruction of serotonergic nerve terminals paralleled the decreases in 5-HT levels in both regions of the brain.

#### Effects of anpirtoline in mice treated with 5,7-DHT

The two-way ANOVA (lesion  $\times$  treatment) on the immobility time showed a significant effect of lesion (sham or 5,7-DHT;  $F_{1,45} = 19.64$ ,  $p < 0.001$ ) and treatment (saline or anpirtoline;  $F_{2,45} = 20.81$ ,  $p < 0.001$ ), as well as a significant interaction between these 2 factors ( $F_{2,45} = 4.27$ ,  $p < 0.05$ ). In the sham-operated mice, anpirtoline was devoid of antidepressant-like activity at 4 mg/kg ( $p = 0.161$ ), but induced an



**Fig. 2:** Effects of treatment with 5,7-DHT on (A) serotonin (5-HT) levels and (B,C) [ $^3$ H]-citalopram binding sites in the cortex and hippocampus of 129/Sv mice ( $n = 7$ –12 per group). We performed one-way analysis of variance (ANOVA) followed by a Newman–Keuls test. †††Statistically significant differences between sham-operated and lesioned mice ( $p < 0.001$ ).

antidepressant-like effect at 8 mg/kg ( $p < 0.05$ ). In mice treated with 5,7-DHT, anpirtoline induced a significant decrease in the immobility time at both doses ( $p < 0.001$  at 4 and 8 mg/kg). For each dose of anpirtoline, the decrease in the immobility time that we observed was significantly greater in the mice treated with 5,7-DHT than in the sham-operated mice ( $p < 0.01$  at 4 mg/kg and  $p < 0.001$  at 8 mg/kg) (Fig. 3).

The two-way ANOVA performed on the locomotor activity showed a significant effect of lesion (sham or 5,7-DHT;  $F_{1,53} = 7.99$ ,  $p < 0.01$ ) and treatment (saline or anpirtoline;  $F_{2,53} = 5.72$ ,  $p < 0.01$ ), but we observed no significant interaction between these 2 factors ( $F_{2,45} = 0.52$ ,  $p = 0.60$ ). Anpirtoline induced a significant increase in the locomotor activity of mice treated with 5,7-DHT at 4 mg/kg ( $p < 0.05$ ). The increase in the number of light beams broken was significantly greater among mice treated with 5,7-DHT than in the sham-operated mice that received 4 mg/kg of anpirtoline ( $p < 0.05$ ) (data not shown).

Taken together, these results suggest that the increased swim attempts of mice treated with 5,7-DHT that received 4 mg/kg of anpirtoline (compared with sham-operated mice) that we observed in the FST could be related to a psychostimulant effect of this drug. However, the absence of psychostimulant effect at 8 mg/kg suggests an enhancement of the antidepressant-like effect of anpirtoline in mice with depleted levels of 5-HT.

#### Effects of local perfusion of anpirtoline

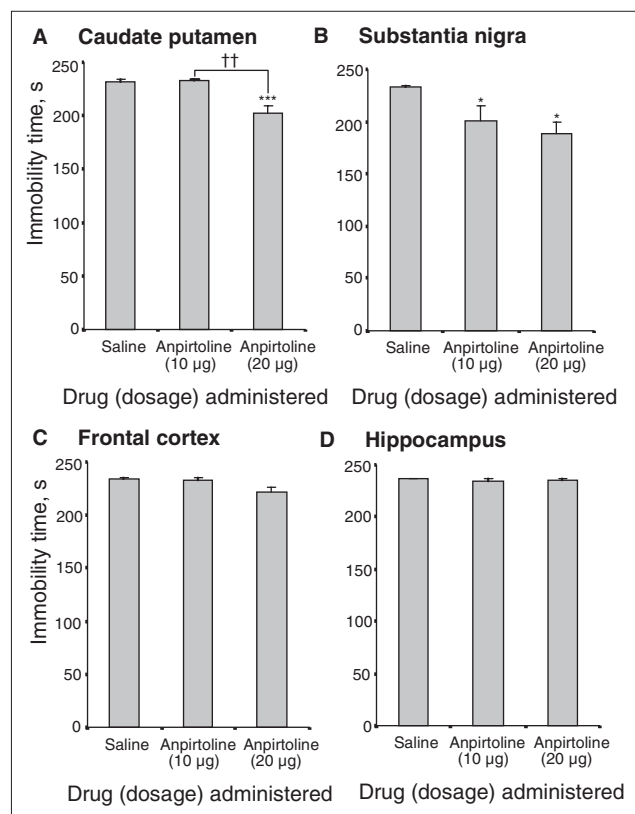
Our one-way ANOVA (treatment factor) revealed a significant effect of anpirtoline ( $F_{2,27} = 10.35$ ,  $p < 0.001$ ) in the cau-



**Fig. 3:** Effects of systemic administration of anpirtoline on the immobility time in the forced swim test (FST) in sham-operated and 5,7-DHT-lesioned 129/Sv mice ( $n = 8-10$  per group). We performed two-way analysis of variance (ANOVA) (lesion  $\times$  treatment) followed by a Newman-Keuls test. Data are expressed as mean and standard error of the mean in seconds. We observed statistically significant differences between mice treated with anpirtoline and those that received saline ( $*p < 0.05$ ,  $***p < 0.001$ ). We observed statistically significant differences between sham-operated and lesioned mice for the same treatment dose ( $\dagger\dagger p < 0.01$ ,  $\dagger\dagger\dagger p < 0.001$ ).

date putamen. The immobility time of mice was significantly reduced following the infusion of the higher dose of the 5-HT<sub>1B</sub> receptor agonist (20  $\mu$ g,  $p < 0.001$ ). Moreover, the antidepressant-like effect was significantly different between the groups treated with 10 and 20  $\mu$ g ( $p < 0.01$ ) (Fig. 4A). In addition, one-way ANOVA revealed a significant effect of anpirtoline ( $F_{2,27} = 4.59$ ,  $p < 0.05$ ) in the substantia nigra. The immobility time of mice was significantly reduced following the infusion of both doses of the 5-HT<sub>1B</sub> receptor agonist ( $p < 0.05$ ). We observed no difference between the 2 treated groups (Fig. 4B).

Conversely, our one-way ANOVA did not reveal any significant effect of anpirtoline ( $F_{2,27} = 0.72$ ,  $p = 0.50$ ) in the frontal cortex, suggesting that infusion of anpirtoline in the prefrontal cortex did not change the immobility time of mice in the FST (Fig. 4C). Furthermore, one-way ANOVA did not reveal a significant effect of anpirtoline ( $F_{2,27} = 0.65$ ,  $p = 0.53$ ) in the hippocampus. The perfusion of anpirtoline in the hippocampus did not induce an antidepressant-like effect in mice in the FST



**Fig. 4:** Effects of the local infusion of anpirtoline on the immobility time in the forced swim test (FST) in Swiss mice after injection in (A) the caudate putamen, (B) the substantia nigra, (C) the frontal cortex or (D) the hippocampus. We performed one-way analysis of variance (ANOVA) followed by a Newman-Keuls test ( $n = 10$  per group). Data are expressed as mean and standard error of the mean in seconds. We observed statistically significant differences between mice treated with anpirtoline and those that received saline ( $*p < 0.05$ ,  $***p < 0.001$ ). We observed statistically significant differences between groups receiving 2 different doses of anpirtoline ( $\dagger\dagger p < 0.01$ ).

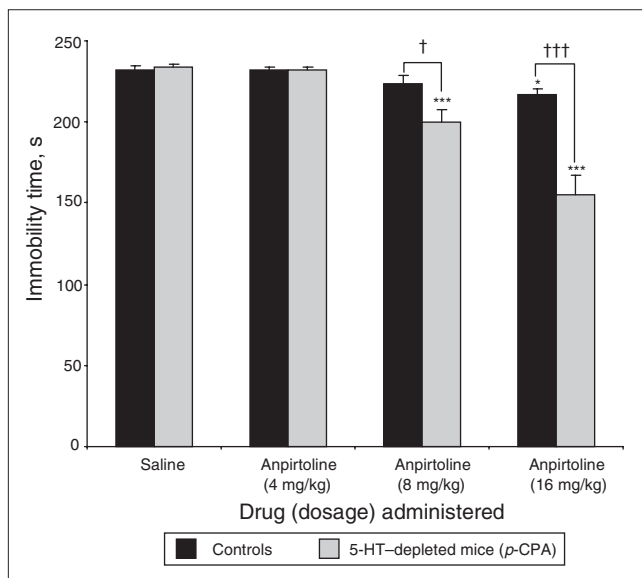
(Fig. 4D). Thus anpirtoline exerts an antidepressant-like effect when injected in regions of the brain that contain DA.

#### Effects of anpirtoline in controls and mice treated with *p*-CPA

The two-way ANOVA on the immobility time showed a significant effect of depletion ( $F_{1,72} = 18.82, p < 0.001$ ) and treatment (saline or anpirtoline;  $F_{3,72} = 28.17, p < 0.001$ ), as well as a significant interaction between these 2 factors ( $F_{3,72} = 7.84, p < 0.001$ ). In control mice, anpirtoline was devoid of antidepressant-like activity at 4 and 8 mg/kg ( $p > 0.05$ ) but induced an antidepressant-like effect at 16 mg/kg ( $p < 0.05$ ). In mice treated with *p*-CPA, anpirtoline induced a significant decrease in the immobility time at 8 and 16 mg/kg ( $p < 0.001$ ). For the 2 higher doses of anpirtoline, the decrease in the immobility time that we observed was significantly greater in mice treated with *p*-CPA than in the control mice ( $p < 0.05$  at 8 mg/kg and  $p < 0.001$  at 16 mg/kg) (Fig. 5).

The two-way ANOVA on the locomotor activity of mice did not reveal statistically significant effects of pretreatment or treatment, nor did it reveal a significant interaction between these 2 factors (data not shown).

Taken together, these results suggest that the increase in the swim attempts of mice treated with *p*-CPA that received 8 and 16 mg/kg of anpirtoline (compared with control mice) that we observed in the FST could be linked to an enhancement of the antidepressant-like effect of anpirtoline in mice with depleted levels of serotonin.



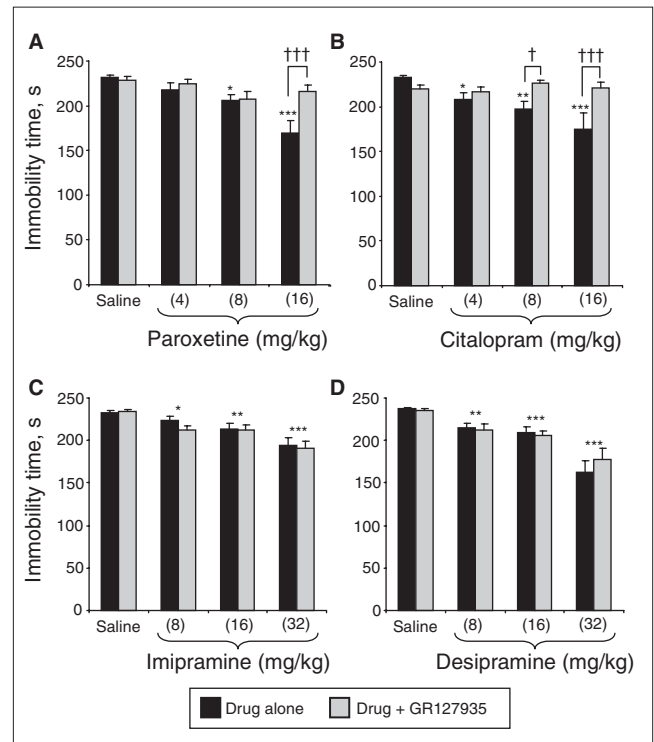
**Fig. 5:** Effects of serotonin (5-HT) depletion, using *p*-CPA, on the antidepressant-like effect of anpirtoline in the forced swim test (FST) in Swiss mice ( $n = 10$  per group). We performed two-way analysis of variance (ANOVA) (depletion  $\times$  treatment) followed by a Newman–Keuls test. Data are expressed as mean and standard error of the mean in seconds. We observed statistically significant differences between mice treated with anpirtoline and those that received saline ( $*p < 0.05$ ,  $***p < 0.001$ ). We observed statistically significant differences between controls and mice with depleted serotonin for the same treatment dose ( $†p < 0.05$ ,  $†††p < 0.001$ ).

#### Effects of combined administration GR127935 and antidepressant

When administered alone, GR127935 (4 mg/kg) was devoid of effect on the immobility time of mice compared with those that received saline. However, we observed different results when it was administered with other drugs.

#### Paroxetine

We tested a range of doses of paroxetine (4, 8 and 16 mg/kg) coadministered with GR127935 (or saline) in the FST. The two-way ANOVA analysis on the immobility time revealed a significant effect of pretreatment (GR127935 or NaCl:  $F_{1,72} = 5.21, p < 0.05$ ) and treatment (paroxetine or NaCl:  $F_{3,72} = 8.84, p < 0.001$ ), as well as a significant interaction between these 2 factors ( $F_{3,72} = 4.19, p < 0.01$ ). Paroxetine administered alone significantly reduced the immobility time for the 2 higher doses tested ( $p < 0.05$  at 8 mg/kg and  $p < 0.001$  at 16 mg/kg), which indicates an antidepressant-like effect. In contrast, the combination of GR127935 and paroxetine



**Fig. 6:** Effects of GR127935 (serotonin 1B [5-HT<sub>1B</sub>] receptor antagonist, 4 mg/kg) on the activity of (A) paroxetine, (B) citalopram, (C) imipramine and (D) desipramine evaluated in the forced swim test (FST) in Swiss mice ( $n = 10$  per group). We performed two-way analysis of variance (ANOVA) (pretreatment  $\times$  treatment) followed by a Newman–Keuls test. Data are expressed as mean and standard error of the mean in seconds. We observed statistically significant differences between treated mice and those that received saline ( $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ). We observed statistically significant differences between controls and mice pretreated with GR127935 for the same treatment dose ( $†p < 0.05$ ,  $††p < 0.01$ ,  $†††p < 0.001$ ).

was devoid of antidepressant-like effect. We found that GR127935 significantly antagonized the behavioural effect of 16 mg/kg of paroxetine compared with the corresponding group of mice that received paroxetine alone ( $p < 0.001$ ) (Fig. 6A).

### Citalopram

We tested a range of doses of citalopram (4, 8 and 16 mg/kg) coadministered with GR127935 (saline) in the FST. The two-way ANOVA analysis on the immobility time revealed a significant effect of pretreatment (GR127935 or NaCl:  $F_{1,72} = 9.56$ ,  $p < 0.01$ ) and treatment (citalopram or NaCl:  $F_{3,72} = 3.91$ ,  $p < 0.05$ ), as well as the interaction between the 2 factors ( $F_{3,72} = 4.68$ ,  $p < 0.05$ ). All doses of citalopram given alone significantly reduced the immobility time ( $p < 0.05$  at 4 mg/kg,  $p < 0.01$  at 8 mg/kg and  $p < 0.001$  at 16 mg/kg). GR127935 alone or coadministered with citalopram was devoid of antidepressant-like effect. At 8 and 16 mg/kg of citalopram, GR127935 significantly antagonized the antidepressant-like effects ( $p < 0.05$  at 8 mg/kg and  $p < 0.001$  at 16 mg/kg) (Fig. 6B).

### Imipramine

We tested a range of doses of imipramine (8, 16 and 32 mg/kg) coadministered with GR127935 (saline) in the FST. The two-way ANOVA analysis on the immobility time revealed a significant effect of treatment ( $F_{3,72} = 23.878$ ,  $p < 0.001$ ). It revealed no significant effect of pretreatment ( $F_{1,72} = 0.09$ ,  $p = 0.76$ ) or significant interaction between the 2 factors ( $F_{3,72} = 0.60$ ,  $p = 0.62$ ). At 8, 16 and 32 mg/kg, imipramine significantly reduced the immobility time ( $p < 0.05$  at 8 mg/kg,  $p < 0.01$  at 16 mg/kg and  $p < 0.001$  at 32 mg/kg). We observed no differences between groups that received imipramine alone and those that received a combination of imipramine and GR127935 (Fig. 6C).

### Desipramine

We tested a range of doses of desipramine (8, 16 and 32 mg/kg) combined with GR127935 (saline) in the FST. The two-way ANOVA analysis on the immobility time revealed a significant effect of treatment ( $F_{3,72} = 15.58$ ,  $p < 0.001$ ), but not of pretreatment ( $F_{1,72} = 0.86$ ,  $p = 0.36$ ), nor of the interaction ( $F_{3,72} = 0.39$ ,  $p = 0.76$ ). All doses of desipramine significantly reduced the immobility time ( $p < 0.01$  at 8 mg/kg and  $p < 0.001$  at higher doses). We observed no differences between groups that received desipramine alone and those that received a combination of desipramine and GR127935 (Fig. 6D).

The administration of GR127935 (4 mg/kg) before citalopram, desipramine and imipramine did not significantly modify the locomotor activity of mice compared with antidepressants administered alone. We obtained a decrease in the locomotor activity when we coadministered GR127935 with 16 mg/kg of paroxetine ( $p < 0.05$ ). We used antidepressants at doses that were devoid of psychostimulant activity (data not shown).

Thus, the pharmacologic blockade of 5-HT<sub>1B</sub> receptor abolished the antidepressant-like effects of SSRIs but not those of desipramine and imipramine.

## Discussion

The antidepressant-like effect of anpirtoline in wild-type mice and the absence of such an effect in 5-HT<sub>1B</sub> knockout mice indicate that the anti-immobility activity of this drug in the FST is specifically linked to the activation of 5-HT<sub>1B</sub> receptors, despite an affinity of anpirtoline for 5-HT<sub>1A</sub>, 5-HT<sub>2</sub> and 5-HT<sub>3</sub> receptor subtypes that has already been described.<sup>24</sup> The systemic administration of anpirtoline (4 mg/kg) induced a statistically significant decrease in the immobility time of wild-type mice evaluated in the FST without affecting their locomotor activity. This decrease should thus be considered as an antidepressant-like effect of anpirtoline.

We destroyed brain 5-HT neurons by 5,7-DHT to evaluate behavioural consequences of the activation of 5-HT<sub>1B</sub> heteroreceptors (5-HT<sub>1B</sub> autoreceptors being destroyed by the lesion of serotonin-containing nerve terminals). Evaluation of monoamine levels showed that 5,7-DHT induced a partial, but significant, depletion of 5-HT (42% in the cortex and 76% in the hippocampus). These 5-HT reductions paralleled the impairment of the high-affinity 5-HT uptake site in several areas of the brain, as measured by autoradiography with [<sup>3</sup>H]-citalopram in mice (−42% v. −45% in the cortex and −76% v. −78% in the hippocampus). In addition, it appears that the lesion was selective, since norepinephrine and DA tissue levels in the cortex and hippocampus were not significantly altered by 5,7-DHT lesion. Interestingly, 5-HT turnover (5-HIAA:5-HT ratio) was not significantly different between sham-operated and lesioned mice in the cortex and hippocampus. These results obtained in mice are different from those described previously,<sup>25</sup> in which the 5-HIAA:5-HT ratio in the hippocampus significantly increased following intracerebroventricular injection of 5,7-DHT in rats. The reasons for these discrepancies are still unclear. However, it is possible that a compensatory increase in the synthesis and release of 5-HT from nerve terminals occurred in rats, but not in mice. Moreover, some lesion studies performed with 5,7-DHT in rats revealed no differences in basal levels of extracellular 5-HT evaluated by microdialysis,<sup>26</sup> whereas others revealed significant differences.<sup>27</sup> It is most likely that these different parameters (brain tissue depletion, extracellular monoamines levels) are linked to compensatory mechanisms such as upregulation of receptors and/or variation of its intrinsic activity.

Similar to the results of the present study, it has been previously demonstrated<sup>28,29</sup> that treatment with the neurotoxin 5,7-DHT did not increase the basal locomotor activity of animals. In our study, mice treated with neurotoxin were also able to swim, as previously described.<sup>30,31</sup>

The lower active dose of anpirtoline in the FST appears, however, to be different between nonoperated and sham-operated mice (4 mg/kg v. 8 mg/kg), which suggests that the absence of effect with anpirtoline might be explained by the isolation of mice for 2 weeks after 5,7-DHT injection. Indeed, it has been previously demonstrated that isolation decreases the antidepressant-like effect of an acute dose of either a SSRI or anpirtoline.<sup>32</sup> On the other hand, the antidepressant-like effect of anpirtoline at a dose of 4 mg/kg persisted in mice treated with 5,7-DHT. Moreover, at both



doses, anpirtoline was more effective in mice treated with 5,7-DHT than in sham-operated mice. The 5,7-DHT lesion seems to potentiate the effect of anpirtoline (by 15% at 4 mg/kg and by 18% at 8 mg/kg). This increase in the antidepressant-like effect of anpirtoline could be explained by compensatory mechanisms enabling remaining 5-HT nerve terminals to maintain basal neurotransmitter activity in mice treated with 5,7-DHT. Accordingly, either an upregulation of 5-HT<sub>1B</sub> heteroreceptors<sup>33</sup> or an increase in their intrinsic activity has been described in 5,7-DHT-lesioned rats.<sup>34,35</sup> The latter explanation is more likely accurate because an upregulation of 5-HT<sub>1B</sub> receptors appears only in rats with 95% depletion of brain 5-HT,<sup>21</sup> whereas we observed a depletion of about 70% brain 5-HT in mice. Considering the lower level of 5-HT depletion that we observed, we could not exclude the possibility that the antidepressant-like effect that we observed in mice treated with 5,7-DHT was at least in part mediated by the activation of 5-HT<sub>1B</sub> autoreceptors located in the cortex and/or in the hippocampus.

To eliminate the latter possibility, we performed a second set of experiments. Because the 129/Sv mouse strain was not suitable for the behavioural test used in these studies,<sup>36</sup> we used a Swiss strain to increase the validity of the results. In these experiments, we demonstrated that the activation of 5-HT<sub>1B</sub> receptors located in both the cortex and hippocampus through local perfusion of anpirtoline does not induce an antidepressant-like effect in the FST. Thus if some 5-HT<sub>1B</sub> autoreceptors could remain active following a 5-HT lesion with 5,7-DHT (because 5-HT lesion is only partial), the antidepressant-like effect observed in the FST is not caused by the activation of 5-HT<sub>1B</sub> autoreceptors located in those areas of the brain. This is in line with the current findings showing that high 5-HT depletion after treatment with *p*-CPA (100% in the hypothalamus and the hippocampus, 96% in the striatum and 83% in the cortex<sup>18</sup>) induces an augmentation of the antidepressant-like effect of anpirtoline evaluated in the FST. After intraraphe injections of 5,7-DHT in rats, an optimal 5-HT depletion (more than 95%) was associated with a large increase in 5-HT<sub>1B</sub> receptor binding in the substantia nigra.<sup>21</sup> Thus, it is possible that a reduced number of presynaptic terminal 5-HT<sub>1B</sub> autoreceptors, as well as somatodendritic 5-HT<sub>1A</sub> autoreceptors, restrained the release of 5-HT. The increased sensitivity of postsynaptic 5-HT<sub>1B</sub> heteroreceptors might also influence the potentiation of the antidepressant-like effects of anpirtoline following the infusion of 5,7-DHT or *p*-CPA. This latter explanation is reinforced by our results, which show that the activation of 5-HT<sub>1B</sub> receptors in areas of the brain that contain high levels of DA (substantia nigra and caudate putamen) and probably of 5-HT<sub>1B</sub> heteroreceptors induces an antidepressant-like effect in Swiss mice.

Since GR127935 is a selective 5-HT<sub>1B</sub> receptor antagonist,<sup>37</sup> our results provide evidence that the antimobility effect of GR127935 coadministered with SSRIs is linked only to its activity on 5-HT<sub>1B</sub> receptors and could not be associated with an activity on 5-HT<sub>1A</sub> receptors. Although the 5-HT<sub>1A</sub> receptor agonists flesinoxan and 8-OHDPAT inhibit the firing rate of dorsal raphe nucleus (DRN) 5-HT neurons, they also produce

a dose-dependent increase in the firing rate of dopaminergic neurons in both the ventral tegmental area and the frontal cortex.<sup>38–40</sup> However, previous studies have reported that pharmacologic (WAY 100635 and NAN 190) or constitutive inactivation of 5-HT<sub>1A</sub> receptors did not antagonize the antidepressant-like effect of SSRIs (paroxetine, citalopram and fluvoxamine) in the FST.<sup>14,41</sup> Interestingly, in a study by Guilloux and colleagues,<sup>41</sup> the swim duration increased, which suggests that the increase in the firing of DA neurons following the activation of 5-HT<sub>1A</sub> receptors is not essential to mediate the antidepressant-like effect of SSRIs in the FST. However, the mixed 5-HT<sub>1A/1B</sub> receptor antagonist pindolol antagonized the antidepressant-like effect of paroxetine in 5-HT<sub>1A</sub> receptor knockout mice, thus supporting the putative impact of 5-HT<sub>1B</sub> receptor activation in the FST.<sup>41</sup> Considering that 5-HT<sub>1B</sub> autoreceptor blockade enhances the neurobiological effects of antidepressants,<sup>2,3</sup> it is suggested that the opposite result obtained in our behavioural study could be linked to the activity of GR127935 on heteroreceptors. It is still not clear why 5-HT<sub>1B</sub> receptor blockade antagonizes the antidepressant-like effect of SSRIs and not those of imipramine and desipramine. The most likely explanation would be the involvement of norepinephrine in the behavioural effect of imipramine and desipramine. It is also important to note that such differences between SSRIs and other antidepressants have already been observed. Indeed, it has been demonstrated that a dopaminergic lesion antagonizes the effects of SSRIs but not those of desipramine and imipramine.<sup>8</sup> This suggests that those drugs do not possess the same mechanism of action for their antidepressant-like effects.

Taken together, our results demonstrate that the activation of 5-HT<sub>1B</sub> heteroreceptors, but not that of autoreceptors, induces an antidepressant-like effect in the FST in mice. It also suggests that the antidepressant-like effect of SSRIs in the FST is caused by the activation of 5-HT<sub>1B</sub> heteroreceptors, which are located in both the caudate putamen and substantia nigra. Since the depletion of DA<sup>8</sup> and 5-HT<sub>1B</sub> receptor blockade inhibits the antidepressant-like effect of SSRIs, it may be that the indirect activation of 5-HT<sub>1B</sub> heteroreceptors by SSRIs induces an increase in dopaminergic neurotransmission and that such an increase is probably responsible for the antidepressant-like effect of SSRIs in the FST in mice.

**Competing interests:** None declared for Drs. Chenu, David, Leroux-Nicollet, Le Maître and Gardier. Dr. Bourin has received honoraria from Lundbeck, Servier and Wyeth, as well as travel assistance from Lundbeck and Servier.

**Contributors:** Drs. Chenu, Gardier and Bourin designed the study. Drs. Chenu, David, Leroux-Nicollet and Le Maître acquired the data, which Drs. Chenu, David, Gardier and Bourin analyzed. Drs. Chenu, Gardier and Bourin wrote the article, which Drs. David, Leroux-Nicollet, Le Maître, Gardier and Bourin reviewed. All authors gave final approval for publication.

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